Lipid Extraction Protocol

1. Lyophilize:

* Turn on Lyophilizer refrigerator
* Freeze, put samples in lyophilizer and apply vacuum.
* Open valve, turn off vacuum, remove samples.
* Weigh to get dry weight, count number of flies, put in bead beating tubes, store at -20C.

1. Add solvents:

* Turn on Centrifuge, temp set to 4C.
* Prep: Add ChCl3 +BHT, MeOH, and H2O (0.5% NaCl) to beakers from stocks, keep ChCl3 and MeOH in hood on ice.
* Pipet 1 mL ChCl3 +BHT into glass vial on ice, close caps tightly.
* Pipet internal standards into vials, keep on ice with caps closed tightly.
* Bead beat dried flies for 20 seconds at speed 4.
* Pipet 1 mL MeOH into bead beating tube.
* Bead Beat for 20 seconds at speed 5.
* Transfer contents of bead beating tubes to glass vials containing 1 mL ChCl3 +BHT and internal standards, return vials to ice with caps closed tightly.
  + Be sure to shake tubes before transferring so content doesn’t get stuck in bottom of tube.
  + Transfer by carefully pouring tube into glass vial
* Vortex each vial for 30s, return to ice.
* Place ice bucket containing vials on shaker for 10 minutes.
* Add 0.9 mL H2O 0.5% NaCl
* Vortex for 15s.
* Place ice bucket containing samples on shaker for 3 minutes.
* Centrifuge at 2000xg at 4C for 5 minutes.

1. Remove Lower Phase

* Label 9mL glass vial for lipids of each sample.
* Remove the bottom phase and pipet in 9mL glass vials.
  + Be careful not to pipet protein layer into new vials.
* Dry under stream of N2 gas in hood until dry.
* Re-suspend in 1 mL 2:1 MeOH:CHCl3
* Freeze at -20C until ready to analyze.